Seed Protein Content of Australian Species of *Acacia*

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MURRAY, D. R., & MCGEE, C. M. Seed protein content of Australian species of *Acacia*. *Proc. Linn. Soc. N.S.W.* 108 (3), (1985) 1986: 187-190.

Seed samples of 26 species of *Acacia* have been milled and extracted with 5% (w/v) potassium sulphate in 0.1M sodium phosphate buffer, pH 7.0, in the presence of Polyclar AT (0.3g per g of meal). The protein concentration of the extracts was determined with a reliable assay involving the biuret reaction. The values obtained for extractable protein content ranged from 4.89% of seed weight *(Acacia victoriae)* to 14.27% *(A. alata)*.

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INTRODUCTION

Acacia seeds may represent an under-utilized human food resource, especially the seeds of Australian species, as these are free of the serious neurotoxins found among the non-protein amino acids of African and Asian species (Evans *et al.*, 1977). Recently, members of the University of Sydney Human Nutrition Unit claimed that the seeds of nine species of *Acacia* eaten by Aborigines were 'strikingly nutrient rich with higher energy, protein and fat content than crops such as wheat and rice and even higher than some meats' (Anonymous, 1984).

In contrast, Murray *et al.* (1978) reported that the amount of protein extracted from seeds of *Acacia sophorae* (Labill.) R.Br. and *A. longifolia* (Andrews) Willd. accounted for only 12 to 13% of seed dry matter content. Analyses have now been performed for a total of 26 species of *Acacia*, including nine considered to be edible (e.g. see Cribb and Cribb, 1976: 78).

MATERIALS AND METHODS

Seed samples of eight edible species of *Acacia* were supplied by Mr M. L. Farrar of Nowra, N.S.W.: *A. adsurgens, A. aneura, A. cowleana, A. coriacea, A. holosericea, A. bivenosa, A. murrayana* and *A. victoriae*. Seeds of *A. tetragonophylla* were provided by Mr J. R. Maconochie of the Northern Territory Herbarium, Alice Springs. Seeds of *A. alata* were collected (by D.R.M.) in the Kooringal Forest south of Perth, W.A.

Seeds of *A. binervata, A. riceana, A. obtusifolia* and *A. triptera* were purchased from Flamingo Enterprises, Nowra, N.S.W. Seeds of *A. floribunda, A. implexa, A. lasiocarpa, A. leioderma, A. mitchellii* and *A. oxycedrus* were from Nindethana Seed Service, Narrikup, W.A. Seeds of the remaining species were from the same batches as recorded in Weder and Murray (1981) and Murray *et al.* (1978). Seed specimens from all the species studied have been retained and will be lodged with the new Wollongong Herbarium.

Extraction Procedure

Seeds (3g to 5g) were finely milled in an electric coffee grinder. Samples of meal (1.0g) were weighed and combined with 0.3g of insoluble polyvinyl pyrrolidone (Polyclar AT) as a precaution against interference by phenolic compounds from seed coats, then suspended in 10ml of 5% (w/v) K_2SO_4 in 0.1M Na phosphate, pH 7.0 (Murray, 1979). Each suspension was stirred continuously for 1h at room temperature, then squeezed through two layers of cheesecloth and centrifuged at 12,000*g* for 20 min. The volume of

recovered supernatant was measured and three aliquots (50μl or 100μl) were removed for protein estimation.

Almost all of the extractable protein is removed with a single extraction step (Murray *et al.*, 1978: 763), but for the samples of edible species supplied by Mr M. L. Farrar, a second extraction was performed by grinding the squeezed material in a mortar with a further 10ml of extraction medium. This homogenate was squeezed through cheesecloth and centrifuged as before. The two supernatants were pooled, mixed thoroughly, and sampled for protein determination as above.

Protein Determination

The aliquots removed for protein determination were treated with ethanol (final concentration 80%, v/v). The resulting precipitates were collected by centrifugation and washed once by resuspension to remove soluble amino nitrogen. The precipitates were collected again by centrifugation, redissolved in 0.25M NaOH, and treated with biuret reagent (Gornall *et al.*, 1949). The absorbance of each tube was read at 540nm. Bovine serum albumin, treated to remove lipids, was used as a reference standard (Collier and Murray, 1977; Murray *et al.*, 1978; Murray, 1979).

Results and Discussion

The extractable protein contents of the seeds of 26 species of *Acacia* are presented in Table 1. As a proportion of seed weight, the protein content ranged from as little as 4.89% *(A. victoriae)* to as much as 14.27% *(A. alata)*. Values for protein content around 10% are common, but it is not possible to conclude that high, medium or low seed protein contents are related to the systematic treatment of an individual species. Of the species considered to be edible, *A. sophorae* (Cribb and Cribb, 1976) had the highest seed protein content (Table 1, and compare Table 5 of Murray *et al.*, 1978).

The biuret method of estimating protein content has been chosen deliberately. The biuret reaction depends upon a property shared by all polypeptide chains — the possession of adjacent pairs of peptide bonds. The procedure is admirably suited to mixtures of different proteins, as a single standard protein is sufficient, and the outcome is independent of differences in amino acid composition between individual proteins.

In contrast, the Kjeldahl method for determining total nitrogen content is far too often applied uncritically to studies of plant proteins, a situation that has not altered since the beginning of this century (Petrie, 1908). In the case of bean seeds *(Phaseolus vulgaris)* a Kjeldahl nitrogen value in mg multiplied by 6.25 yields an apparent protein content that is an overestimate by a factor of two (Adriaanse *et al.*, 1969). An even greater disparity has been observed for seeds of *Cuscuta reflexa*, where a protein estimation based on Kjeldahl nitrogen determination exceeds the seeds' content of extractable protein by 5.7-fold (Rahman and Krishnan, 1971).

Petrie (1908) was probably the first person to study the nitrogenous constituents of *Acacia* seeds, and he determined that as much as 45% of the total nitrogen of mature *A. pycnantha* seeds occurs as non-protein nitrogen. Total reliance on the Kjeldahl procedure has thus led to overestimation of *Acacia* seed protein content by the group referred to previously (Anonymous, 1984). Their estimate of 24% protein for immature *A. cowleana* seeds must be compared with a value of 10.42% for mature seeds of this species (Table 1). The range of 17% to 27% protein content for seeds of all the species in this group's sample is clearly extravagant. We question their view that 'many bushfoods appear to be richer sources of nutrients than similar cultivated plants' (Anonymous, 1984). The quantity, the quality and the availability of proteins from seeds are all factors that need to be assessed. At this stage little is known about any of these properties for the seed

TABLE 1

Extractable Protein Content of Acacia *Seeds*

Species	Mean seed mass (mg)	Protein content: mg per seed	% of seed weight
Botryocephalae			
A. decurrens Willd.	13.1	1.35	10.34
A. elata A. Cunn. ex Benth.	43.2	3.69	8.55
Uninerves: Racemosae			
A. bivenosa DC[1,2]	34.9	2.53	7.24
A. murrayana F. Muell. ex Benth.[1]	29.4	2.01	6.84
A. victoriae Benth.[1]	26.6	1.30	4.89
Phyllodinous Species of Indistinct Alliance			
A. binervata DC[3]	20.6	1.72	8.35
A. tetragonophylla F. Muell.	30.7	4.06	13.23
Plurinerves			
A. coriacea DC[1]	70.1	4.82	6.88
A. implexa Benth.	20.8	1.80	8.67
A. melanoxylon R.Br. ex Ait.	15.2	1.21	7.94
Juliflorae (with Spicatae)			
A. adsurgens Maiden & Blakely[1]	7.9	0.68	8.61
A. floribunda (Vent.) Willd.	7.1	0.69	9.72
A. longifolia (Andr.) Willd.[4]	24.6	3.20	13.02
A. sophorae (Labill.) R.Br.[1,5]	37.7	4.70	12.47
A. obtusifolia A. Cunn.	14.2	1.63	11.50
A. oxycedrus Sieb. ex DC	19.6	1.77	9.01
A. riceana Henslow	9.9	0.99	10.03
A. triptera Benth.	11.1	1.21	10.90
A. aneura F. Muell. ex Benth.[1]	18.2	1.97	10.82
A. cowleana Tate[1]	11.5	1.20	10.42
A. holosericea A. Cunn. ex G. Don.[1]	11.4	1.06	9.32
Pulchellae and *A. alata*			
A. alata R.Br.[6]	10.0	1.43	14.27
A. drummondii Lindl.	2.66	0.31	11.66
A. lasiocarpa Benth.	4.52	0.44	9.81
A. leioderma Maslin	5.26	0.61	11.61
Other Bipinnatae			
A. mitchellii Benth.[6]	14.3	1.49	10.41

1 considered edible.
2 = *A. ligulata*, see Pedley (1979).
3 see Tindale and Roux (1969, 1974).
4 freshly milled sample from batch L1(2), Murray *et al.* (1978).
5 freshly milled sample from batch S3, Murray *et al.* (1978).
6 for discussion, see Guinet *et al.* (1980), Murray and Weder (1983).

proteins of native legumes. It may be assumed that the contents of essential sulphur-containing amino acids in *Acacia* seed globulins are very low, as values for these amino acids were not included in the analyses reported by Pettigrew and Watson (1975). The best-balanced protein sources from seeds are to be found among the albumin fractions of some of the cultivated legumes (for review, see Murray, 1984a,b).

Detailed electrophoretic studies on the seed proteins of the *Acacia* species listed in this paper will be the subject of a future communication.

ACKNOWLEDGEMENTS

We thank all those who supplied seeds, the Joyce W. Vickery Scientific Research Fund (Linnean Society of N.S.W.) and the Australian Biological Resources Study for financial support. Thanks are also due to F. Vairinhos, Miss J. Plummer and Mrs M. Cordova-Edwards for assistance (1981, 1982) and Prof. J. S. Pate for hospitality during a visit to Perth by D.R.M. in 1982.

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